GRASAS Y ACEITES 69 (1)
January-March, 2018, e235
ISSN-L: 0017-3495
https://doi.org/10.3989/gya.0671171

Enrichment of Cinta Senese burgers with omega-3 fatty acids. Effect of type of addition and storage conditions on quality characteristics

C. Aquilani[a, ✉], T. Pérez-Palacios[b], F. Sirtori[a], E. Jiménez-Martín[b], T. Antequera[b], O. Franci[a], A. Acciaioli[a], R. Bozzi[a] and C. Pugliese[a]

[a]Department of Agrifood Production and Environmental Sciences, School of Agriculture, University of Florence, Italy.
[b]Food Science, School of Veterinary Science, University of Extremadura, Spain

✉Corresponding author: chiara.aquilani@unifi.it

Submitted: 26 June 2017; Accepted: 30 October 2017

SUMMARY: The most beneficial omega-3 PUFAs to human health, EPA and DHA fatty acids, are typically present in fish products, but extraneous to meat. Therefore, Cinta Senese pork burgers were added with microencapsulated (M) and bulk fish oil (F) and subjected to three storage conditions: no storage (T0), chilled (T5) and frozen storage (T30). The physico-chemical and sensory attributes of raw and cooked burgers were investigated. After storage and cooking, EPA and DHA were better preserved in M burgers than in F samples, which showed the highest TBAR values at T0 and T5, while M samples presented scores similar to the control. Panelists observed differences mainly in greasy appearance, odor intensity and cooked meat odor and flavor. The M group showed the best scores at T5 with respect to the control and F burgers. So, fish oil microencapsulation was an effective method to prevent EPA and DHA oxidation while respecting burger quality characteristics.

KEY WORDS: *Fish oil; Meat quality; Microencapsulation; Pork; Sensorial attributes*

RESUMEN: ***Enriquecimiento de la hamburguesa Cinta Senese con ácidos grasos omega-3. Efecto del tipo de adición y condición de almacenamiento en las características de calidad.*** EPA y DHA son los ácidos grasos poliinsaturados omega 3 más beneficiosos para la salud humana, se presentan típicamente en el pescado, y no se encuentran en carnes. Por ello, se elaboraron hamburguesas de cerdo de la especie "Cinta Senese" añadiendo aceite de pescado (F), microcápsulas que contenían aceite de pescado (M) o sólo a base de carne (control (C)) y se mantuvieron bajo las siguientes condiciones de almacenamiento: sin almacenaje (T0), en refrigeración (T5) y congelación (T30). Se estudiaron los atributos sensoriales y físico-químicos de las hamburguesas crudas y cocinadas. En cuanto al almacenamiento y el cocinado, las hamburguesas con microcápsulas preservaron mejor los EPA y DHA que las muestras con aceite de pescado, las cuales presentaron los valores más altos de TBARs en las muestras T0 y T5, mientras que las M mantuvieron unos resultados similares a las de tipo control. En los resultados de la cata realizada se observaron, entre los tratamientos realizados y respecto a las distintas condiciones de almacenamiento, diferencias en la apariencia grasienta, olor y flavor a carne cocida e intensidad de olor. En las hamburguesas M se obtuvieron las mejores puntuaciones frente a las encontradas en F y C en el tipo T5. Por tanto, la microencapsulación de aceite de pescado se verificó como un método efectivo para prevenir la oxidación de EPA y DHA respetando la calidad y características de las hamburguesas.

PALABRAS CLAVE: *Aceite de pescado; Calidad de la carne; Características organolépticas; Cerdo; Microencapsulación*

ORCID ID: Aquilani C https://orcid.org/0000-0001-6282-0993, Pérez-Palacios T https://orcid.org/0000-0002-0095-5785, Sirtori F https://orcid.org/0000-0002-7443-0156, Jiménez-Martín E https://orcid.org/0000-0002-2767-0413, Antequera T https://orcid.org/0000-0001-6951-0015, Franci O https://orcid.org/0000-0003-4517-7371, Bozzi R https://orcid.org/0000-0001-8854-0834, Acciaioli A https://orcid.org/0000-0002-4395-0423, Pugliese C https://orcid.org/0000-0002-3735-8714

Citation/Cómo citar este artículo: Aquilani C, Pérez-Palacios T, Sirtori F, Jiménez-Martín E, Antequera T, Franci O, Bozzi R, Acciaioli A, Pugliese C. 2108. Enrichment of Cinta Senese burger with omega-3 fatty acids. Effect of type of addition and storage condition on quality characteristics. *Grasas Aceites* **69** (1), e235. https://doi.org/10.3989/gya.0671171

1. INTRODUCTION

Alpha-linolenic acid (ALA C18:3 n-3) and its longer-chain metabolites, i.e. eicosapentaenoic acid (EPA C20:5 n-3) and docosahexaenoic acid (DHA C22:6 n-3), are important polyunsaturated fatty acids (PUFAs), due to their role in the prevention and treatment of cardiovascular diseases, some types of cancer and immune inflammatory diseases (Garcia-Almeida *et al.,* 2010; Pelliccia *et al.,* 2013; Dienke *et al.,* 2016). However, the real intake of these PUFAs in traditional Western diets is far below the recommended minimum of 250 mg per day of long-chain omega-3 PUFAs (Sanders, 2000). Indeed, the main sources of EPA and DHA are oily fish, oil fish supplements and to a lesser degree white fish and shellfish, which are hardly appreciated in most Western countries. Consequently, many studies focused on improving the nutraceutical quality of widespread foods by increasing their omega-3 content.

It is worth noting that the food selected to be enriched should be well accepted, popular, inexpensive and easy to cook. Moreover, it should constitute a potential opportunity to valorize products for the food industry, i.e. by adding value to less commonly accepted or traditional food. Diet habits are changing in accordance with a lifestyle that is focused on time-saving, and consequently, there is a growing demand for "ready-to-heat" products, such as burger meats. The popularity of these convenience products makes them a promising strategy to increase the intake of omega-3 fatty acids using enrichment methods.

Among animal products, meat and meat products appear to be an interesting target to be enriched because of their high consumption and fatty acid profile. Indeed, they are characterized by a low content of long-chain omega-3 PUFAs together with a high presence of monounsaturated fatty acids (approximately 45-50%) and saturated fatty acids (approximately 45–55%) (Givens *et al.*, 2006). Fortification can be carried out by feeding the animals with omega-3 enriched feedstuff (Corino *et al.*, 2014) or by enriching the products through a technological approach. In this last case, three main ways can be identified. The simplest one is directly adding fish (or vegetal) oil to food (Càceres *et al.*, 2008; Valencia *et al.*, 2008; Martínez *et al.*, 2012); another method is oil emulsification (Salminen *et al.,* 2013), which, in contrast to the former, provides PUFAs protection from lipid oxidation during the product's shelf-life, but it is unable to mask undesirable odors and flavors (mainly in the case of fish oil) (Jiménez-Colmenero, 2007). The last method consists of encapsulating the oil emulsion to form a single (or multi-) layer around each oil drop. This method is more complex, but it ensures the best results in preventing oxidation, preserving food sensorial attributes and avoiding the perception of fish or rancid flavors (Jiménez-Colmenero, 2007; Josquin *et al.,* 2012; Keenan *et al.,* 2015).

To meet consumer demand for healthier and more widely accepted meat products, burgers have been chosen for enrichment with fish oil as omega-3 source. The fortification could constitute an opportunity to re-valorize some products, such as Cinta Senese ones. This is a local pig breed reared extensively in Tuscany; its meat has obtained the Protected Designation of Origin (PDO) and has good perspectives for increasing its relevance. Its production is mainly focused on dry-cured products, above all hams and salami (Pugliese and Sirtori, 2012). Fresh meat from Cinta Senese pigs has not achieved a large diffusion in the market, which is likely related to the consumers' association of Cinta Senese only with dry-cured products and to the perception of fresh meat as unhealthy due to its high lipid content and low percentage of PUFA (Pugliese *et al.*, 2005). Therefore, the addition of omega 3 PUFAs to Cinta Senese fresh meat seemed to be an effective way to enlarge the market of Cinta Senese fresh products and to valorize this traditional breed.

The aim of the present study was to investigate the ease of producing omega-3 enriched burgers from Cinta Senese loins, given that their quality traits could be affected by the enrichment procedure (bulk fish oil vs. microencapsulated fish oil) and by the storage method typically used for burgers (chilled or frozen). The development of this type of product would improve the profitability of a local and high-quality pig production system.

2. MATERIALS AND METHODS

2.1. Burger manufacture and sampling

Burgers were made with loins of Cinta Senese pigs provided by Azienda Agricola Borgonovo (Cortona, AR, Italy). Ten pigs were bred outdoors and fed with commercial feed. Ten portions of fresh loins (a total of approximately 11kg of meat) were minced and mixed with salt (2%), sulfites (0.05%) and mashed potato powder (2.4%). Three types of burgers were made: control (C) (3.7 kg of mixture), with no further modifications, microcapsule burgers (M) adding 173 g of microcapsules to 3.7 kg of mixture and fish oil burgers (F) adding 6 g fish oil to 3.7 kg of mixture. The respective quantities of microcapsules and fish oil were calculated to contain the same amount of EPA+DHA(1.67g).

The burgers were made by weighing 90g of mixture and shaping it into a standard burger mould.

For cooked samples, the following cooking procedure was used: grilling at 165 °C, flipping every 2 min until reaching an internal temperature of 73–75 °C, recorded using a thermometer probe (Testo 735-2, Lenzkirch, Germany).

Forty-eight burgers were made for each addition (C, M, F) and used as follows:

Five burgers underwent physico-chemical analysis as fresh matter; five burgers were cooked and used for physico-chemical analyses at 0 days (T0); seven burgers were cooked and underwent sensorial analysis at T0; five burgers were stored at 4 °C for 5 days (T5), then cooked and analyzed; seven burgers were stored under the same conditions (T5), cooked and examined by panelists; five burgers were stored at -20 °C for 30 days (T30), then cooked and analyzed; seven burgers were stored under the same conditions (T30), cooked and examined by panelists.

2.1.1. Physico-chemical analyses of raw and cooked burgers

The determinations carried out were: cooking loss, instrumental color and water activity; fat, protein, moisture content; fatty acid profile; lipid oxidation (TBARs).

A quantitative-descriptive sensorial analysis was carried out on cooked burgers only.

2.2. Fish oil

Fish oil was kindly provided by Biomega Natural Nutrients (Galicia, Spain). It is a low viscosity, vacuum deodorized oil. As reported by the producer, its omega-3 PUFA contents for 100 g of product are 5.96g of EPA and 25.83g of DHA.

2.3. Microcapsules

Multi-layer microcapsules were elaborated following the methodology of Jiménez-Martín *et al.,* (2016a) with some modifications. Fish oil (Biomega Natural Nutrients, Galicia, Spain) was used as a source of omega-3 PUFA (5.96% EPA, 25.83% DHA, 0.02% BHT). The process started from a primary emulsion made of 20 g of fish oil, 6 g of soy lecithin (Biogran S.L., Madrid, Spain) and dissolved in 174 g of water. This mixture was added to a solution made of 2 g of chitosan (Trades, Chitoclear FG 95, Murcia, Spain) dissolved in 198 g of acetic acid (1%) (Scharlau, Barcelona, Spain) to form a secondary emulsion, which was homogenized (Homogenizer SPX, APV2000, Denmark) at 700 bar. This was added to 400 g of water and a maltodextrin (30%) (Roquette, Glucidex 12, Lestrem, French) solution for finally obtaining a feed emulsion. The feed emulsion (800g) was dehydrated and turned into powder using a laboratory-scale spray-dryer (Mini Spray Dryer B-290 Buchi, Switzerland) equipped with a 0.5 mm nozzle atomizer. The aspirator rate was adjusted to 80%, feed rate was 1 L/h, inlet temperature was 180 °C, and outlet temperature ranged 85–90 °C. The obtained microcapsules contained 9.63 mg of EPA+DHA per gram of microcapsules and 4% moisture, which was calculated following the methodology of Jiménez-Martín *et al.,* (2016b).

2.4. Physico-chemical analysis

Fresh (n=15) and cooked (n=45) burgers were first analyzed by means of instrumental color. Then, samples were minced, and water activity and moisture were immediately determined. The rest of sample was stored at -80 ºC until further analysis.

2.4.1. Cooking loss

Cooking loss was calculated as the difference between the weight of cooked and fresh burgers and it was expressed as a percentage.

2.4.2. Instrumental Color

Instrumental color was measured on the surface of the burgers. Fresh samples were examined the same day they were processed. In the case of cooked samples, measurements were taken when they had reached room temperature (20–25 °C). Instrumental color was determined using a Minolta CR-300 colorimeter (Minolta Camera Corp., Meter Division. Ramsey, NJ) with illuminant D65, a 0º standard observer and a 2.5 cm port/viewing area. The following color coordinates were determined: lightness (L^*), redness (a^*) and yellowness (b^*). The colorimeter was standardized before use with a white tile having the following values: L^*=93.5, a^*=1.0 and b^*=0.8.

2.4.3. Water activity

For the water activity, the system Lab Master-aw (NOVASINA AG, Switzerland) was used after calibration.

2.4.4. Moisture, fat content and protein

The moisture in fresh and cooked samples was determined gravimetrically at 100 ± 2 ºC by the official method (A.O.A.C., 2000a, reference 935.29).

Fat content was determined gravimetrically with chloroform:methanol (2:1, vol/vol), following the method described by Pérez-Palacios *et al.,* (2008).

Protein content was calculated in duplicate by the Kjeldahl method (A.O.A.C., 2000b, reference 992.15).

2.4.5. Fatty acids

Fatty acid methyl esters (FAMEs) from extracted fat were prepared by basic transesterification following the official method (A.O.A.C., 2000c, reference

963.22), using hexane and hydroxide potassium 2N. FAMEs were analyzed by gas-chromatography (GC) using a Hewlett–Packard HP-5890A gas chromatograph, equipped with an on-column injector and a flame ionization detector (FID), using a polyethylene glycol capillary column (Supelcowax-10, Supelco, Bellefonte, PA, USA) (60 m × 0.32 mm i.d. × 0.25 μm film thickness). The GC oven program temperature was as follows: initial temperature of 180 °C was raised at 5 °C/min to 200 °C, kept at this temperature for 40 min, raised at 5 °C/min to 250 °C, and then kept for an additional 21 min. The injector and detector temperatures were 250 °C. The carrier gas was helium at a flow rate of 0.8 ml/min. Individual FAME peaks were identified by comparison of their retention times with those of standards (Sigma, St. Louis, MO, USA). Peak areas were measured and FAMEs were expressed as area percentage of total area FAMEs (%).

2.4.6. Lipid oxidation

Thiobarbituric acid-reactive substances (TBARS) were measured following the extraction method described by Salih *et al.,* (1987). Each burger was minced in a kitchen blender, and 2.5 g were homogenized for 2 min with 7.5 mL of 3.86% perchloric acid and 0.25 mL of butylated hydroxytoluene (4.2%). The tubes were kept on ice to avoid heat degradation. This homogenate was filtered and centrifuged (4 min, 3500 rpm). The supernatant (2 mL) was mixed with 2 mL of thiobarbituric acid 0.02 M. At the same time, a standard curve was prepared employing 1,1,3,3- tetraethoxypropane (TEP). Immediately, the mixture was heated to 90 ºC for 30 min, cooled and centrifuged again (2 min, 3500 rpm). Absorbance was measured at 532 nm on a spectrophotometer (Hitachi U-2000, Tokyo, Japan). The concentration of malonaldehyde (MDA) was calculated from the standard curve, developed simultaneously with the samples using solutions of TEP (Merck, Schchardt, Germany). TBARS were expressed as mg MDA kg^{-1} sample.

2.5. Sensory analysis

All sessions were done in sensory panel rooms with the conditions specified in the UNE regulation (Norma UNE, 1979) equipped with white fluorescent lighting (220-230 V, 35 W). A piece of cooked burger (20g) was served hot on white plastic plates to panelists, marked with random three-digit codes. The panel sessions were held around 1-2 h before lunch time. Salt-free crackers and a glass of water at room temperature were provided to each panelist to rinse between samples. The burgers were assessed by a trained panel of 18 members using a descriptive analysis method. Eleven sensory traits grouped under appearance (greasy appearance), odor (odor intensity, cooked meat odor) texture (hardness, juiciness, oiliness), taste (salty) and flavor (cooked meat flavor, rancid flavor, flavor intensity, after taste) were assessed. Selected subjects underwent further training in meat and meat products sensory characteristics over five years. The number of burgers used for sensorial analysis was 7 for each supplementation, repeated for T0, T5 and T30. Each burger was divided into four parts to be served to panelists for a total of 28 pieces of burgers of each type (C, M, and F). Each panelist evaluated three pieces of burger in each session, and the sample order was randomized across assessors. Sensory traits were assessed by panelists in a 10 cm unstructured line, ranging from "less" to "more".

2.6. Statistical analysis

The effect of cooking and type of enrichment were analyzed by two-way ANOVA using SAS. (1996) SAS/STAT software, release 9.4. When significant differences were observed ($p < 0.05$), they were evaluated by a Tukey's test. The following model was used:

$$Y_{ijk} = \mu + T_i + A_j + e_{ijk}$$

Where: Y is the j^{th} observation, μ is the overall mean, T is the i^{th} treatment; A is the j^{th} addition and e_{ij} is the error, which is an independent random variable. The interaction between factors was tested, but it resulted not significant for any variable.

For the sensorial data the following model was used:

$$Y_{ijkl} = \mu + A_i + P_j + T_k + e_{ijkl}$$

Where: Y is the j^{th} observation, μ is the overall mean, A is the i^{th} addition; P is the j^{th} panelist, T is the k^{th} treatment and e_{ijk} is the error, which is an independent random variable.

3. RESULTS AND DISCUSSION

Table 1 shows values for physico-chemical and instrumental color parameters in fresh and cooked Cinta Senese burgers. In fresh meat, significant differences between treatments were found for moisture and protein. The values for C burger were in agreement with previous studies on Cinta Senese meat (Pugliese *et al.*, 2005). Regarding differences between M and F burgers, they cannot be supported by other scientific evidence, since this trial was the first study on Cinta Senese burger omega-3 enrichment. Some physico-chemical and color changes were found in fresh burgers, where the F group had the highest L*value, in accordance with Martínez *et al.,* (2009). This can be due to the presence of oil which increased the lightness. As observed for

TABLE 1. Physio-chemical parameters and instrumental color in fresh and cooked Cinta Sense burgers as affected by type of omega-3 enrichment

		Fresh burger				Cooked burger					
		C	M	F	*p* (*addition*)	C	M	F	*p* (*addition*)	SEM	*p* (cooking)
Water activity		0.95^{b}	0.95^{b}	0.96^{a}	**	0.96^{a}	0.95^{b}	0.96^{a}	**	0.000	n.s.
Moisture (%)		58.44^{abx}	57.33^{bx}	59.51^{ax}	**	56.59^{ay}	54.47^{by}	56.89^{ay}	***	0.605	***
Fat (%)		14.11	13.05	14.01	n.s.	13.65	12.44	13.15	n.s.	2.140	n.s.
Protein (%)		22.09^{a}	20.74^{b}	21.64^{a}	**	22.00^{a}	20.25^{b}	20.24^{b}	**	0.454	n.s.
Cooking loss (%)		-	-	-	-	11.41^{ab}	13.00^{a}	9.70^{b}	**		-
Instrumental color	***L****	58.78^{bx}	60.21^{abx}	61.27^{ax}	**	51.46^{y}	51.12^{y}	52.92^{y}	n.s.	3.906	***
	a*	17.90^{x}	17.27^{x}	17.78^{x}	n.s.	9.66^{y}	10.26^{y}	9.43^{y}	n.s.	0.933	***
	b*	12.08^{y}	12.08^{y}	12.66^{y}	n.s.	16.90^{x}	17.37^{x}	16.92^{x}	n.s.	1.439	***

[a]Means and mean standard errors (SEM) for fresh and cooked burgers with no enrichment (C), enriched with micro-encapsulated fish oil (M) and with bulk fish oil (F).
[b]Different letters (a,b,c) within the same treatment indicate significant differences within addition (control, micro-capsule or fish oil); different letters (x,y) in the same line indicate significant differences between treatments (fresh vs. cooked) within the same addition.

physico-chemical and instrumental color parameters, the type of omega-3 enrichment significantly affected water activity, moisture and protein content. In fresh and cooked burgers, the M samples showed the lowest water activity, moisture and protein values. These effects can be explained by the addition of extra dry matter due to the incorporated microencapsulation material. Thus, in M burgers, 4.7% fish oil microcapsules were added, which contain around 4% moisture, with the rest of the 96% sample being dry matter (Jiménez-Martín *et al*., 2014). Josquin *et al*., (2012) found the same effect, with around 10% lower moisture content in sausages with encapsulated oil than in those with pure fish oil. After cooking, the M burgers showed significantly higher cooking loss than C and F ones; this result could also explain the lowest moisture value found in M burgers after cooking. Heck *et al*., (2017), studied pork burgers added with microencapsulated vegetal oil and observed a higher cooking loss value in control samples compared to modified ones. The type of enrichment did not change the fat content in fresh burgers, as values were similar in the three batches (C, M and F). So, the amount of fish oil added, either microencapsulated or not, was not high enough to cause changes in the total amount of burger fat. In cooked samples, again, M sample showed the lowest moisture, water activity and protein, while they were the most affected by cooking loss. No significant differences were observed due to the type of addition among cooked burgers.

The values for moisture, *L** and *a** significantly decreased from fresh to cooked burgers, while b* levels increased. These modifications were expected and in agreement with previous studies (Baggio *et al.*, 2006), which also reported a significant decrease in the moisture in beef burgers after grilling: while Martínez *et al.,* (2012), observed that moisture, *L** and *a** values were significantly lower in cooked burger patties than in fresh ones. As expected, the lipid content was not modified by the cooking procedure since no fat source was used to cook, as reported also by Baggio *et al.,* (2006) for different grilled meat products.

The results of the physico-chemical analysis with respect to storage and type of addition are shown in Table 2. After chilled storage (T5), moisture and water activity followed the same trend of T0, being higher in M samples than in C and F. As for T0, cooking loss was also found to be higher in M samples with respect to the F ones. Regarding T0, a significant difference was found for L* which was highest in F samples. This is in accordance with results reported by Martínez *et al*., (2012) in hamburgers and by Valencia *et al*., (2008) in fresh pork sausages, where modified samples after a week of chilled storage showed the highest L* scores. After frozen storage none of the above-mentioned parameters were affected by addition, except for a*, which was lower in F samples. Within the same treatment, C and M samples showed higher values for moisture, water activity and a lower cooking loss, compared to T0 burgers.

The fatty acid composition of fresh and cooked Cinta Senese burgers from C, M and F batches is displayed in Table 3. The general profile of fatty acids was similar in all samples. MUFAs were the major family of fatty acids, followed by SFAs and with PUFAs being the minor one. Oleic acid (C18:1 n-9) showed the highest percentage, followed, in decreasing order, by palmitic (C16:0), stearic (C18:0), linoleic (C18:2 n-6), palmitoleic (C16:1 n-7) and myristic (C14:0) acids; the rest of fatty acids showed percentages lower than 1%. This is in agreement with other studies on Cinta Senese lipid composition (Pugliese *et al.*, 2005). Moreover, no

TABLE 2. Physico-chemical parameters and instrumental color in cooked Cinta Senese burgers as affected by storage (T0 = no storage, T5 = chilled storage, T30 = frozen storage) and type of omega-3 enrichment (C = Control, M = microcapsules and F = fish oil)

		Addition					p (storage)			p (storage * addition)
	Storage	C	M	F	SEM	p (addition)	C	M	F	
Moisture (%)	T0	56.59^{ay}	54.47^{by}	56.89^{a}	0.469	*	*	*	n.s.	n.s.
	T5	56.97^{axy}	55.26^{by}	57.24^{a}		*				
	T30	57.87^{x}	57.65^{x}	57.46		n.s.				
Water activity	T0	0.96^{ay}	0.96^{by}	0.96^{a}	0.001	**	*	*	n.s.	n.s.
	T5	0.96^{ax}	0.96^{bxy}	0.96^{a}		**				
	T30	0.96^{xy}	0.96^{x}	0.96		n.s.				
Cooking loss (%)	T0	11.41^{bx}	13.00^{ax}	9.70^{cxy}	0.497	*	n.s.	**	*	*
	T5	10.82^{axy}	9.59^{abxy}	8.51^{by}		**				
	T30	9.57^{y}	10.59^{y}	10.49^{x}		n.s.				
L*	T0	51.46	51.12	52.92	0.980	n.s.	n.s.	n.s.	n.s.	n.s.
	T5	51.30^{b}	52.42^{b}	55.27^{a}		*				
	T30	52.75	52.39	53.12		n.s.				
a*	T0	9.66	10.26	9.43	0.893	n.s.	n.s.	n.s.	n.s.	n.s.
	T5	9.80	8.85	8.53		n.s.				
	T30	9.12^{ab}	11.39^{a}	8.49^{b}		*				
b*	T0	16.90	17.37	16.92	0.681	n.s.	n.s.	n.s.	n.s.	n.s.
	T5	15.51	15.80	15.48		n.s.				
	T30	16.13	17.17	16.24		n.s.				
Fat (%) on a wet basis	T0	13.65	12.44	13.15	0.427	n.s.	n.s.	n.s.	n.s.	n.s.
	T5	14.23	13.46	14.15		n.s.				
	T30	13.56	12.93	13.28		n.s.				
Protein (%) on a wet basis	T0	20.71^{y}	20.25^{y}	20.24^{y}	0.245	n.s.	***	**	***	n.s.
	T5	20.76^{y}	20.25^{y}	20.73^{y}		n.s.				
	T30	22.60^{x}	22.45^{x}	22.74^{x}		n.s.				

[a] Means and mean standard errors (SEM) for burgers with no enrichment (C), enriched with micro-encapsulated fish oil (M) and with bulk fish oil (F), cooked after different storage conditions (T0=no storage, T5=chilled storage, T30=frozen storage).
[b] Different letters in the same row (a, b, c) indicate significant differences (at least $p < 0.05$) through addition within the same storage conditions.
[c] Different letters in the same column (x, y, z) indicate significant differences (at least $p < 0.01$) regarding storage.

important modifications in the percentages of fatty acids were observed during the cooking procedure. This indicates that the fatty acids in Cinta Senese loin are not very susceptible to change due to the cooking conditions applied in the present study. In different meat products, Baggio *et al.,* (2006) found no modification in fatty acid profiles after grilling, whereas Martínez *et al.,* (2012), reported significant differences in myristolenic (C14:1), arachidonic (C20:4 n-6) and DHA (C22:6 n-3) acids contained in grilled beef burgers compared to raw samples.

Concerning the enrichment-type influence on fatty acid composition, as expected, in C burgers (fresh and cooked) EPA and DHA were not found; while in M fresh and cooked burgers, significantly higher percentages of EPA and DHA were observed respect to F ones. Since the same omega-3 quantity (1.67 g) has been added in M and F batches, data indicate that maltodextrin and chitosan, which constitute the microcapsule outer layer, provided an effective protection to omega-3 added PUFAs both during manufacturing and cooking. In contrast, Keenan *et al.,* (2015) and Josquin *et al.,* (2012) found a significant increase in the total amount of PUFAs in both encapsulated and bulk fish oil samples. This difference can be explained considering that, in these studies, microcapsules and fish oil were used as a partial replacement of pork back fat; while, in the present work, the fortification was carried out without a previous modification of lipid meat content. Partially according with the studies previously reported, M burgers also showed higher n-3 and lower n-6/n-3 ratio than F and C ones in cooked samples. Indeed, both Keenan *et al*., (2015) and Josquin *et al*., (2012) observed a lower n-6/n-3 ratio in omega-3 enriched samples, but the way fish

TABLE 3. Fatty acid composition (expressed as percentage of fatty acid methyl esters) in fresh and cooked Cinta Senese burgers as affected by type of omega-3 enrichment.

	Fresh burger				Cooked burger					
%	C	M	F	*p* (*addition*)	C	M	F	*p* (*addition*)	SEM	*p* (cooking)
C12	0.11	0.11	0.11	n.s.	0.12	0.11	0.11	n.s.	0.001	n.s.
C14	1.82	1.96	1.97	n.s.	2.04	1.93	1.84	n.s.	0.059	n.s.
C14:1	0.04	0.04	0.04	n.s.	0.04^{ab}	0.03^{b}	0.05^{a}	n.s.	0.000	n.s.
C15	0.05	0.05	0.06	n.s.	0.05	0.06	0.05	n.s.	0.000	n.s.
C16	27.91	28.48	29.46	n.s.	29.86	28.77	28.56	n.s.	1.679	n.s.
C16:1	3.14	3.15	3.03	n.s.	3.25	3.13	3.08	n.s.	0.057	n.s.
C17	0.21	0.22	0.23	n.s.	0.22^{b}	0.22^{ab}	0.22^{a}	n.s.	0.000	n.s.
C17:1	0.22	0.20	0.20	n.s.	0.21	0.21	0.21	n.s.	0.000	n.s.
C18	10.45	10.96	11.64	n.s.	10.89	11.28	11.31	n.s.	1.258	n.s.
C18:1 n-9	43.55	42.42	41.28	n.s.	41.54	41.84	42.31	n.s.	1.690	n.s.
C18:2 n-6	10.29	10.01	9.72	n.s.	9.75	9.91	9.93	n.s.	0.170	n.s.
C18:3 n-6	0.02	0.02	0.03	n.s.	0.02	0.03	0.03	n.s.	0.000	n.s.
C18:3 n-3	0.56	0.54	0.52	n.s.	0.52^{b}	0.54^{a}	0.54^{ab}	n.s.	0.001	n.s.
C20	0.12	0.13	0.14	n.s.	0.12	0.13	0.13	n.s.	0.001	n.s.
C20:1	0.70	0.73	0.67	n.s.	0.61	0.74	0.71	n.s.	0.007	n.s.
C20:2	0.37	0.36	0.34	n.s.	0.32	0.36	0.37	n.s.	0.001	n.s.
C21	0.07	0.05	0.07	n.s.	0.06	0.07	0.07	n.s.	0.000	n.s.
C20:4 n-6	0.32	0.34	0.31	n.s.	0.33	0.39	0.37	n.s.	0.000	n.s.
C20:3 n-3	0.07	0.07	0.07	n.s.	0.06	0.07	0.07	n.s.	0.000	n.s.
C20:5 n-3	ND^{b}	0.07^{a}	0.05^{a}	***	ND^{c}	0.07^{a}	0.03^{b}	***	0.000	n.s.
C22:6 n-3	ND^{b}	0.09^{a}	0.06^{ab}	**	ND^{b}	0.10^{a}	0.03^{b}	***	0.001	n.s.
SFA	40.73	41.96	43.68	n.s.	43.36	42.59	42.28	n.s.	3.371	n.s.
MUFA	47.64	46.53	45.22	n.s.	45.64	45.94	46.36	n.s.	2.026	n.s.
PUFA	11.63	11.51	11.10	n.s.	11.00	11.47	11.37	n.s.	0.243	n.s.
∑n-6	10.63	10.37	10.06	n.s.	10.10	10.33	10.33	n.s.	0.179	n.s.
∑n-3	0.63^{b}	0.77^{a}	0.70^{ab}	**	0.58^{c}	0.78^{a}	0.67^{b}	***	0.003	n.s.
n-6/n-3	16.89^{a}	13.53^{b}	14.52^{b}	***	17.45^{a}	13.29^{c}	15.35^{b}	***	0.630	n.s.
SFA/PUFA	0.69	0.72	0.78	0.181	0.77	0.74	0.73	0.329	0.003	0.459

[a]Means and mean standard errors (SEM) for fresh and cooked burgers with no enrichment (C), enriched with microencapsulated fish oil (M) and with bulk fish oil (F).
[b] Different letters (a,b,c) within the same treatment indicate significant differences after addition (control, micro-capsule or fish oil); different letters (x,y) in the same line indicate significant differences between treatments (fresh vs. cooked) within the same addition.
[c] ND: not detected.

oil was added (bulk or encapsulated) seemed to make no difference.

As occurred with most of the Cinta Senese burger fatty acids, cooking did not significantly influence EPA and DHA percentages. However, in F samples, there was a decreasing tendency of the EPA and DHA percentages from fresh to cooked samples, which was not observed in M burgers. This suggests that microcapsules perform a protective effect on these omega-3 fatty acids during cooking.

Table 4 shows the fatty acid profiles of C, M and F burgers with regard to storage. The influence of storage on the FA profile of the burgers was very limited. At T5, α-linolenic acid (C18:3 n3), arachidonic acid and eicosatrienoic acid (C20:3 n-3) were observed to be the highest in the M samples. At T5, M samples preserved the highest percentage of EPA, DHA and omega-3 FA with respect to both C and F burgers. However, in F samples, frozen storage resulted in a better preservation of EPA, DHA and consequently, the omega-3 total content increased if compared to chilled storage. On the contrary, in M burgers, frozen storage determined a loss in DHA and omega-3 with respect to EPA content, if compared to chilled storage. This is probably due, as suggested by Keenan *et al*., (2015), to a number of possible mechanisms occurring during the spray-drying process which, combined

with the larger surface of microencapsulated fish oil, in comparison to bulk oil, as well as the long-term storage (30 days), could have promoted the omega-3 degradation. Total PUFA contents were also positively modified by addition at T0 and T5, both in M and F burgers, though, due to the limited amount of fish oil added both as microcapsules and bulk oil, the PUFA improvement consisted only in a 0.4-0.8% increase in M samples and 0.2-0.4% in F samples. Nevertheless, thanks to the fish oil FA profile, rich in omega-3 PUFA, a small amount of it was able to increase the omega-3 content of enriched samples and consequently, to significantly reduce the omega6/omega3 ratio averagely from 17 to 12-13 in M samples and to 14-15 in F samples. These scores are still far from the recommendations of a 4/1 ratio (Wood *et al*., 2008), however, combining the omega-3 enrichment with a partial replacement of fat has already shown promising results in reducing SFA meat contents and lowering omega-6/omega-3 ratios (Josquin *et al*., 2012; Keenan *et al*., 2015).

Figure 1 shows the lipid oxidation of fresh versus cooked burgers from C, M and F batches. In fresh samples, no differences in oxidation levels were detected. However, the effect of enrichment type

TABLE 4. Fatty acid composition (expressed as percentage of fatty acid methyl esters) in cooked Cinta Senese burgers as affected by storage (T0 = no storage, T5 = chilled storage, T30 = frozen storage) and type of omega-3 enrichment (C = Control, M = microcapsules and F = fish oil).

		Addition					p (storage)			p (addition * treatment)
	Storage	C	M	F	p (addition)	SEM	C	M	F	
C18	T0	10.89	11.28	11.31	n.s.	0.403	n.s.	n.s.	n.s.	n.s.
	T5	11.12	11.68	11.34	n.s.					
	T30	11.11	11.27	10.59	n.s.					
C18:1	T0	41.54	41.84	42.31^{y}	n.s.	0.572	n.s.	n.s.	n.s.	n.s.
	T5	42.13	43.44	42.86	n.s.					
	T30	42.79	42.64	44.02^{x}	n.s.					
C18:2 n-6	T0	9.75	9.91	9.93	n.s.	0.129	n.s.	n.s.	n.s.	n.s.
	T5	9.88	10.23	9.95	n.s.					
	T30	10.03	10.09	10.18	n.s.					
C18:3 n-6	T0	0.02^{b}	0.03^{ab}	0.03^{a}	*	0.002	n.s.	n.s.	n.s.	n.s.
	T5	0.03	0.02	0.03	n.s.					
	T30	0.03	0.03	0.03	n.s.					
C18:3 n-3	T0	0.52	0.54	0.54	n.s.	0.009	n.s.	n.s.	n.s.	n.s.
	T5	0.53^{b}	0.56^{a}	0.53^{b}	*					
	T30	0.54	0.55	0.55	n.s.					
C20:4 n-6	T0	0.33^{b}	0.39^{a}	0.37^{ab}	*	0.017	n.s.	n.s.	n.s.	n.s.
	T5	0.35^{b}	0.42^{a}	0.37^{b}	**					
	T30	0.37	0.38	0.40	n.s.					
C20:3 n-3	T0	0.06^{y}	0.07^{y}	0.07	n.s.	0.005	n.s.	n.s.	*	n.s.
	T5	0.07^{by}	0.08^{ax}	0.07^{ab}	*					
	T30	0.08^{x}	0.07^{y}	0.08	n.s.					
C20:5 n-3	T0	0.00^{y}	0.07^{a}	0.03^{by}	***	0.005	*	n.s.	*	*
	T5	0.01^{cxy}	0.07^{a}	0.04^{bxy}	***					
	T30	0.02^{bx}	0.07^{a}	0.06^{ax}	***					
C22:6 n-3	T0	0.00^{c}	0.10^{ay}	0.03^{by}	***	0.013	n.s.	n.s.	*	*
	T5	0.00^{c}	0.15^{ax}	0.05^{bxy}	*					
	T30	0.00^{b}	0.10^{ay}	0.08^{ax}	***					
SFA	T0	43.36	42.59	42.28^{x}	n.s.	0.747	n.s.	n.s.	n.s.	n.s.
	T5	42.63	40.60	41.69	n.s.					
	T30	41.72	41.67	40.01^{y}	n.s.					

(Continued)

TABLE 4. (*Continue*) Fatty acid composition (expressed as percentage of fatty acid methyl esters) in cooked Cinta Senese burgers as affected by storage (T0 = no storage, T5 = chilled storage, T30 = frozen storage) and type of omega-3 enrichment (C = Control, M = microcapsules and F = fish oil).

		Addition					p (storage)			p (addition * treatment)
	Storage	C	M	F	p (addition)	SEM	C	M	F	
MUFA	T0	45.64	45.94	46.36^{y}	n.s.	0.586	n.s.	n.s.	n.s.	n.s.
	T5	46.17	47.44	46.89^{y}	n.s.					
	T30	46.83	46.69	48.21^{x}	n.s.					
PUFA	T0	10.99^{by}	11.47^{ay}	11.37^{a}	**	0.173	n.s.	n.s.	n.s.	n.s.
	T5	11.20^{by}	11.96^{ax}	11.42^{ab}	*					
	T30	11.45^{x}	11.65^{x}	11.78	n.s.					
N6	T0	10.10	10.33	10.33	n.s.	0.139	n.s.	n.s.	n.s.	n.s.
	T5	10.25^{b}	10.68^{a}	10.35^{ab}	*					
	T30	10.43	10.49	10.61	n.s.					
N3	T0	0.58^{cx}	0.78^{ay}	0.67^{ay}	***	0.024	*	n.s.	*	*
	T5	0.60^{bxy}	0.86^{ax}	0.69^{by}	***					
	T30	0.64^{bx}	0.78^{ay}	0.77^{ax}	***					
N6/N3	T0	17.45^{ax}	13.29^{cy}	15.35^{bx}	***	0.303	*	n.s.	*	*
	T5	17.04^{axy}	12.43^{cy}	14.99^{bxy}	***					
	T30	16.32^{ay}	13.49^{bx}	13.90^{by}	***					
SFA/UFA	T0	0.77	0.74	0.73^{x}	n.s.	0.021	n.s.	n.s.	n.s.	n.s.
	T5	0.74	0.69	0.72^{x}	n.s.					
	T30	0.72	0.72	0.67^{y}	n.s.					

[a]Different letters in the same raw (a, b, c) indicate significant differences ($p < 0.05$) through addition within the same storage conditions.
[b]Different letters in the same column (x, y, z) indicate significant differences (at least $p < 0.05$) with storage.
[c]The following FAs were detected, but not reported in the table: lauric acid, miristic acid, miristoleic acid, pentadecaenoic acid, palmitic acid, palmitoleic acid, margaric acid, optadecenoic acid, steric acid,arachic acid, gondoic acid and gadoleic acid.

strongly impacted cooked samples, with the highest TBAR values for F samples. M burgers, despite the type of addition, showed similar oxidation levels to C. In accordance with our results, Jiménez-Martín *et al.,* (2016a) observed the highest TBAR values in nuggets fortified with bulk fish oil, while microencapsulated and control ones had significantly lower values. These results were also supported by the lower levels of hexanal and nonanal (two additional lipid oxidation markers) in encapsulated-enriched nuggets. Furthermore, Josquin *et al.,* (2012) working with fish oil, reported the lowest TBAR content in fermented sausages enriched with encapsulated oil compared to those in the control and bulk oil added ones. The treatment, as expected, significantly increased TBARs from fresh to cooked samples in M and F batches. This is due to the high temperature-boosting effect on the oxidation processes taking place during cooking (Valencia *et al.*, 2008; Martínez *et al.*, 2012). Nevertheless, the extent of lipid oxidation was not the same for all batches. The increase in TBAR values from fresh to cooked samples was more marked in the F batch than in the C and M ones, which, again, indicates that microencapsulated omega-3 fatty acids were protected during cooking.

Figure 2 reports the TBAR scores with respect to addition and storage. M samples were the least oxidized, with values similar to C samples, after both chilled and frozen storage. This is in accordance with the observations made by Valencia *et al*., (2008) on fish oil added burgers and by Jiménez-Martín *et al*., (2016a) on chicken nuggets enriched with microencapsulated and bulk fish oil after frozen storage. However, other authors have obtained contrasting results, showing how, in some cases, the spray-drying technique, mainly due to its elevated temperature, could negatively affect the oxidation of the encapsulated fish oil drops (Pelser *et al*., 2007; Keenan *et al*., 2015). Nevertheless, for both M and F, lipid oxidation scores were far below the perception threshold of 2.00 mg MDA/Kg of product reported by Greene and Cumuze (1982) as the detectable level perceivable by the majority of meat consumers.

Results from the quantitative-descriptive sensory analysis of cooked burgers are shown in Figure 3. The type of omega-3 enrichment led to significant differences mainly in the following sensory attributes: greasy appearance, odor intensity, cooked meat odor, oiliness, and cooked meat flavor. At T0 and T30, M group showed the lowest scores with

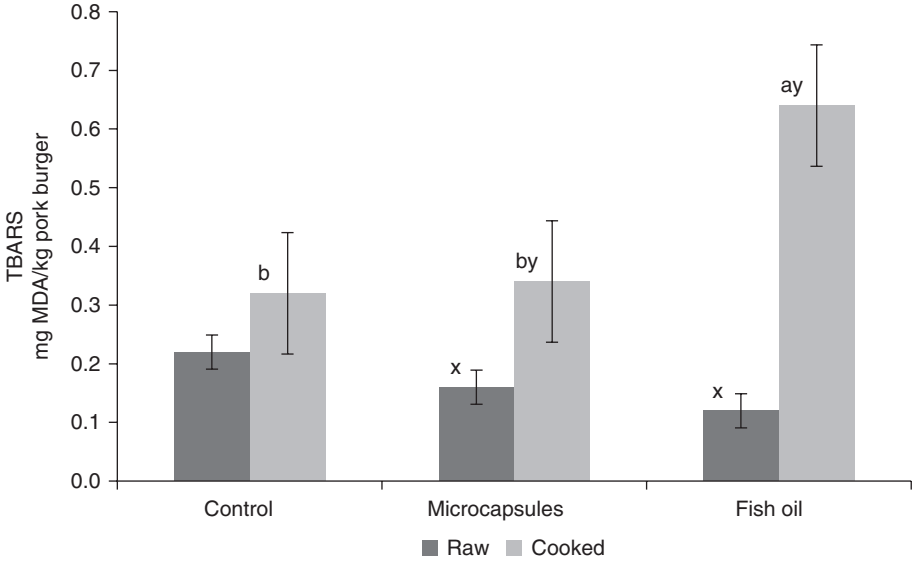

FIGURE 1. Lipid oxidation in raw (■) and cooked (■) Cinta Senese burgers as affected by the omega-3 enrichment type (control, micro-capsules, fish oil).
[a] Different letters (a,b) within the same treatment (raw or cooked) indicate significant effect ($p < 0.05$) of omega-3 enrichment type; different letters (x,y) within the same addition indicate significant effect ($p < 0.05$) of treatment (raw or cooked).

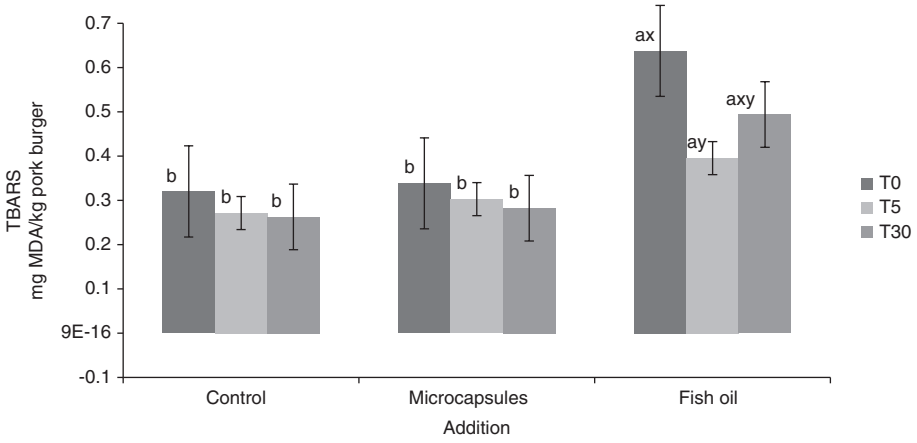

FIGURE 2. Lipid oxidation at T0 (■), T5 (■) and T30 (■) in Cinta Senese burgers as affected by the type of omega-3 enrichment.
[a]Different letters (a, b, c) within the same storage (T0 ■, T5 ■, T30 ■) indicate significant differences (at least $p < 0.05$) among addition types.
[b]Different letters (x, y, z) within the same addition type (Control, Microcapsules, Fish oil) indicate significant differences at least $p < 0.05$) with storage (T0 ■, T5 ■, T30 ■).

respect to C and F burgers. On the contrary, at T5, M burgers showed the best scored for those attributes, while F burgers had the lowest. Very few studies on meat products enriched with microencapsulated fish oil are available. Some authors observed no differences in the sensorial characteristics of fermented sausages (Pelser *et al.*, 2007) or chicken nuggets (Jiménez-Martín *et al.*, 2016a) enriched with microencapsulated oil, neither for sausages (Josquin *et al.*, 2012) or pork burgers (Martínez *et al.*, 2012) added with bulk fish oil. However, in enriched burgers, Keenan *et al.*, (2015) reported the presence of off odors and flavors described as 'fishy' or not 'native' by panelists, who, partially in accordance with our results, gave higher acceptability scores to control samples with respect to added ones, regardless of the type of omega-3 enrichment. In fact, together with the oxidation issue, another pivotal problem linked to the use of fish oil for food enrichment is its peculiar odor and flavor, which, being easily perceivable, can negatively impact the enriched product's sensory characteristics. This problem can be partially avoided since several methods have been developed in order to deodorize the fish oil (Garg *et al.*, 2006)

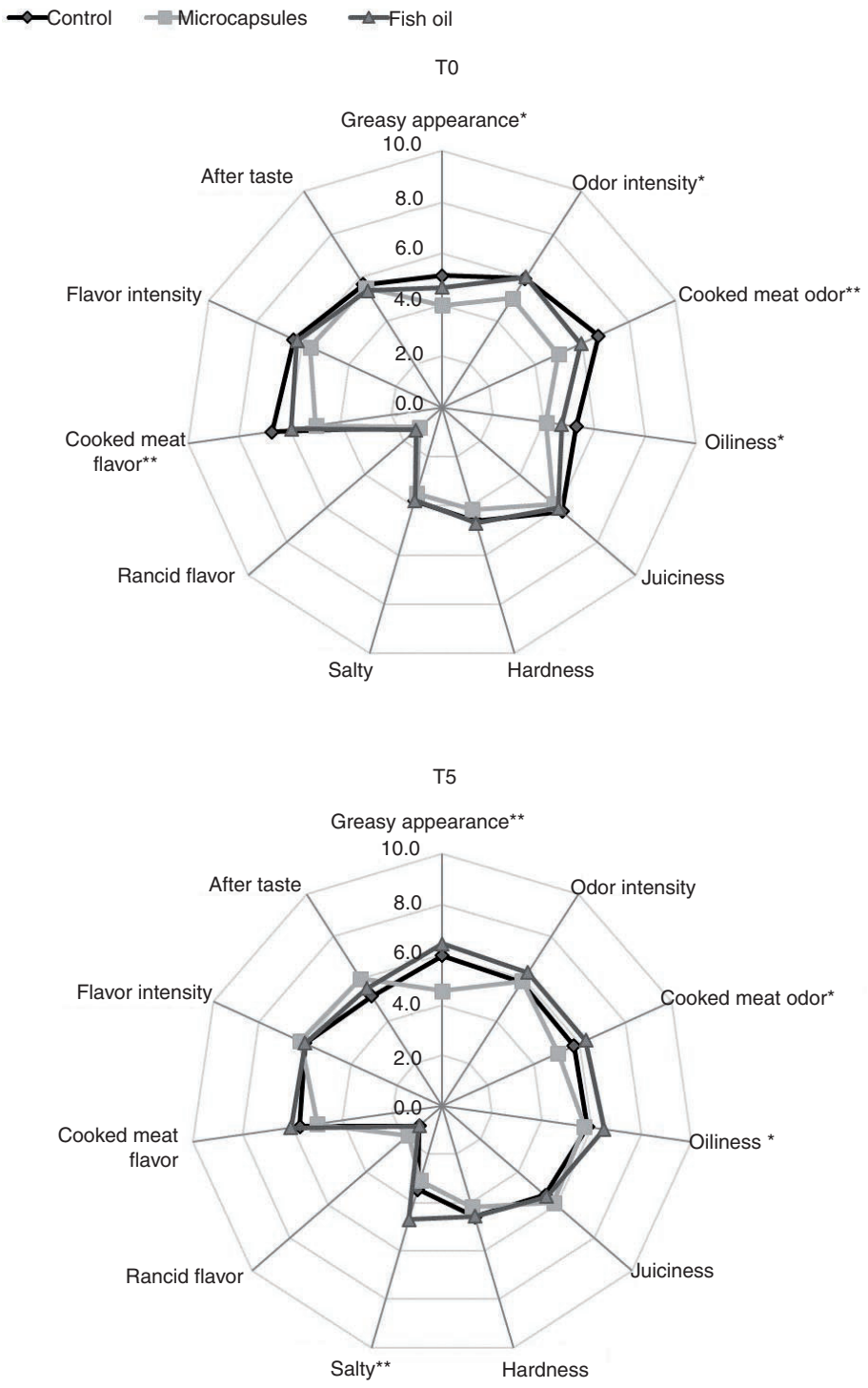

FIGURE 3. (*Continued*)

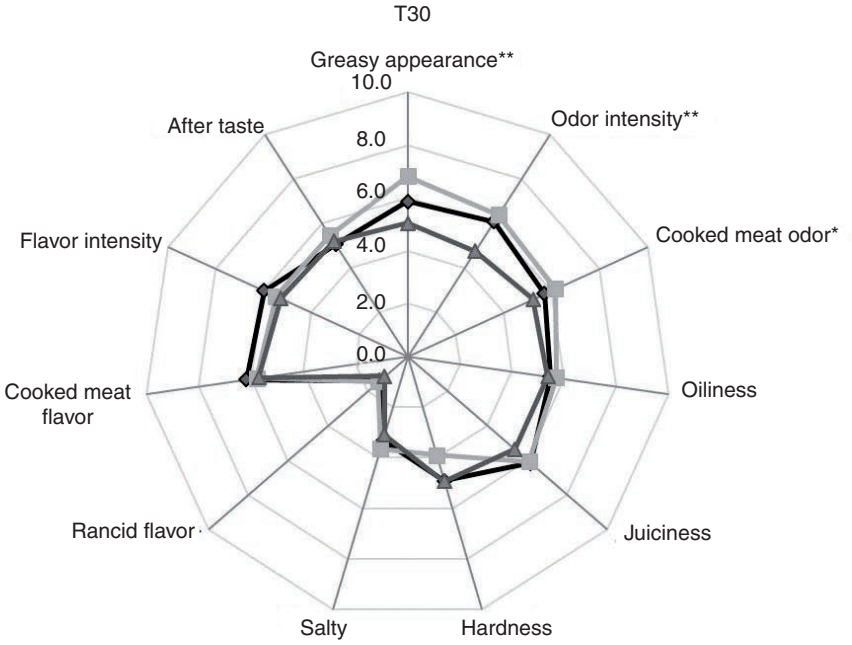

FIGURE 3. Results of the sensory analysis of Cinta Senese cooked burgers as affected by type of omega-3 enrichment (control (◆), micro-capsules (■), fish oil (▲)) at T0, T5 and T30. The asterisks ((*) $p < 0.05$, (**) $p < 0.01$) indicate significant effects of the omega-3 enrichment type on the burgers' sensory characteristics.

so that, in most cases, as well as in this work, enrichment is carried out using deodorized fish oil.

Nevertheless, as reported before, this is not always enough to fully protect the enriched product from fishy aroma and taste.

4. CONCLUSIONS

Fish oil addition, both with bulk and encapsulated fish oil, has been found to be suitable to fortify Cinta Senese burgers with EPA+DHA. M fortification did not affect burger instrumental color, total lipid content or fatty acid profile, except for EPA and DHA. Indeed, their content was found to be greater in M burgers after cooking and after storage with respect to F ones. This is in accordance with lipid oxidation scores, which were comparable for C and M, while F samples were always the most oxidized. Finally, sensorial analyses results indicate that chilled storage is more suitable for products added with microcapsules, whereas, for bulk fish oil enriched burgers, frozen storage is more appropriate. To sum up, multi-layer microcapsules produced by spray-drying were observed to be effective in enriching poorly provided foods with omega-3 PUFAs. In addition, producing omega-3 enriched burgers from Cinta Senese pigs, might improve the profitability of this local and high-quality pig production system.

ACKNOWLEDGEMENTS

This project received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 634476 (Project acronym: TREASURE). The content of this paper reflects only the author's view and the European Union Agency is not responsible for any use that may be made of the information it contains.

REFERENCES

A.O.A.C. 2000a. Moisture in Malt Gravimetric Method. (935.29). In *Official Methods of Analysis* (17th ed.). Gaithersburg, Maryland: Association of Official Analytical Chemist.

A.O.A.C. 2000b. Crude Protein in Meat: Block Digestion Method. (992.15). In *Official Methods of Analysis* (17th ed.). Gaithersburg, Maryland: Association of Official Analytical Chemist.

A.O.A.C. 2000c. Methyl Esters of Fatty Acids in Oils and Fats: Gas Chromatographic Method. (963.22). In *Official Methods of Analysis* (17th ed.). Gaithersburg, Maryland: Association of Official Analytical Chemist.

Baggio SR, Bragagnolo, N. 2006. The effect of heat treatment on the cholesterol oxides, cholesterol, total lipid and fatty acid contents of processed meat products. *Food Chem*. **95**, 611–619. https://doi.org/10.1016/j.foodchem.2005.01.037

Cáceres E, García ML, Selgas MD. 2008. Effect of pre-emulsified fish oil - as source of PUFA n-3 - on microstructure and sensory properties of mortadella, a Spanish bologna-type sausage. *Meat Sci*. **80**, 183–193. https://doi.org/10.1016/j.meatsci.2007.11.018

Corino C, Rossi R, Cannata S, Ratti S. 2014. Effect of dietary linseed on the nutritional value and quality of pork and

pork products: Systematic review and meta-analysis. *Meat Sci*. **98**. https://doi.org/10.1016/j.meatsci.2014.06.041

Dienke BJ, Van Montfort SJT, Oranje B, Durston S, Smeets PAM. 2016. Effects of omega-3 polyunsaturated fatty acids on human brain morphology and function: what is the evidence?. *Eur. Neuropsychopharmacol.* **26**, 546–61. https://doi.org/10.1016/j.euroneuro.2015.12.031

García-Almeida JM, Murri-Pierri M, Lupiéñez Y, Rico-Pérez JM, Saracho-Dominguez H, Roca MM, Garcia-Aleman J, Casado-Ferneńdez G, Medina-Carmona J, Tinahones-Madueño F. 2010. PP299 effect of oral supplementation enriched with omega-3 fatty acids in inflammatory parameters and oxidative stress in patients with otolaryngologist cancer treated with radiotherapy. *Clin. Nutr. Supplements* **5**. https://doi.org/10.1016/S1744-1161(10)70374-4

Garg ML, Wood LG, Singh H, Moughan PJ. 2006. Means of delivering recommended levels of long chain n-3 polyunsaturated fatty acids in human diets. *J. Food Sci*. **71**. https://doi.org/10.1111/j.1750-3841.2006.00033.x

Givens DI, Kliem KE, Gibbs RA. 2006. The role of meat as a source of n-3 polyunsaturated fatty acids in the human diet. *Meat Sci*. **74**, 209–18. https://doi.org/10.1016/j.meatsci.2006.04.008

Greene BE, Cumuze TH. 1982. Relationship Between TBA Numbers and Inexperienced Panelists' Assessments of Oxidized Flavor in Cooked Beef. *J. Food Sci.* **47**, 52–54. https://doi.org/10.1111/j.1365-2621.1982.tb11025.x

Heck RT, Vendruscolo RG, de Araújo Etchepare M, Cichoski AJ, de Menezes CR, Barin JS, Campagnol PCB. 2017. Is it possible to produce a low-fat burger with a healthy n − 6/n − 3 PUFA ratio without affecting the technological and sensory properties? *Meat Sci*. **130**, 16–25. https://doi.org/10.1016/j.meatsci.2017.03.010

Jiménez-Colmenero F. 2007. Healthier lipid formulation approaches in meat-based functional foods. Technological options for replacement of meat fats by non-meat fats. *Trends Food Sci. Technol*. **18**, 567–578. https://doi.org/10.1016/j.tifs.2007.05.006

Jiménez-Martín E, Gharsallaoui A, Pérez-Palacios T, Carrascal JR, Rojas TA. 2014. Suitability of Using Monolayered and Multilayered Emulsions for Microencapsulation of ω-3 Fatty Acids by Spray Drying: Effect of Storage at Different Temperatures. *Food Bioprocess Technol*. **8**, 100–111. https://doi.org/10.1007/s11947-014-1382-y

Jiménez-Martín E, Pérez-Palacios T, Carrascal JR, Rojas TA. 2016a. Enrichment of Chicken Nuggets with Microencapsulated Omega-3 Fish Oil: Effect of Frozen Storage Time on Oxidative Stability and Sensory Quality. *Food Bioprocess Technol*. **9**, 285–297. https://doi.org/10.1007/s11947-015-1621-x

Jiménez-Martín E, Rojas TA, Gharsallaoui A, Carrascal JR, Pérez-Palacios T. 2016b. Fatty acid composition in double and multilayered microcapsules of ω-3 as affected by storage conditions and type of emulsions. *Food Chem*. **194**, 476–486. https://doi.org/10.1016/j.foodchem.2015.08.046

Josquin NM, Linsse JPH, Houben JH. 2012. Quality characteristics of Dutch-style fermented sausages manufactured with partial replacement of pork back-fat with pure, pre-emulsified or encapsulated fish oil. *Meat Sci.* **90**, 81–6. https://doi.org/10.1016/j.meatsci.2011.06.001

Keenan DF, Resconi VC, Smyth TJ, Botinestean C, Lefranc C, Kerry JP, Hamill RM. 2015. The effect of partial-fat substitutions with encapsulated and unencapsulated fish oils on the technological and eating quality of beef burgers over storage. *Meat Sci.* **107**, 75–85. https://doi.org/10.1016/j.meatsci.2015.04.013

Martínez B, Miranda JM, Vázquez BI, Fente CA, Franco CM, Rodríguez JL, Cepeda A. 2012. Development of a Hamburger Patty with Healthier Lipid Formulation and Study of its Nutritional, Sensory, and Stability Properties. *Food Bioprocess Technol.* **5**, 200–208. https://doi.org/10.1007/s11947-009-0268-x

Pelliccia F, Marazzi G, Greco C, Franzoni F, Speziale G, Gaudio C. 2013. Current evidence and future perspectives on n-3 PUFAs. *Int. J. Cardiol*. **170**, S3–S7. https://doi.org/10.1016/j.ijcard.2013.06.044

Pelser WM, Linssen JPH, Legger A, Houben JH. 2007. Lipid oxidation in *n* - 3 fatty acid enriched Dutch style fermented sausages. *Meat Sci*. **75**, 1-11. https://doi.org/10.1016/j.meatsci.2006.06.007

Pérez-Palacios T, Ruiz J, Martín D, Muriel E, Antequera T. 2008. Comparison of different methods for total lipid quantification in meat and meat products. *Food Chem.* **110**, 1025–1029. https://doi.org/10.1016/j.foodchem.2008.03.026

Pugliese C, Sirtori F. 2012. Quality of meat and meat products produced from southern European pig breeds. *Meat Sci*. **90**, 511–8. https://doi.org/10.1016/j.meatsci.2011.09.019

Pugliese C, Bozzi R, Campodoni G, Acciaioli A, Franci O, Gandini G. 2005. Performance of Cinta Senese pigs reared outdoors and indoors. 1. Meat and subcutaneous fat characteristics. *Meat Sci*. **69**, 459–64. https://doi.org/10.1016/j.meatsci.2004.09.001

Salih AM, Smith DM, Price JF, Dawson LE. 1987. Modified extraction 2-thiobarbituric acid method for measuring lipid oxidation in poultry. *Poul. Sci.* **66**, 1483–1488.

Salminen H, Herrmann K, Weiss J. 2013. Oil-in-water emulsions as a delivery system for *n*-3 fatty acids in meat products. *Meat Sci*. **93**, 659–667. https://doi.org/10.1016/j.meatsci.2012.11.035

Sanders TA. 2000. Polyunsaturated fatty acids in the food chain in Europe. *Am. J. Clin. Nutr.* **71**, 176S-8S.

SAS. (1996). SAS/STAT software, release 9.4. SAS Institute Inc., Cary, NC.

Valencia I, O'Grady MN, Ansorena D, Astiasarán I, Kerry JP. 2008. Enhancement of the nutritional status and quality of fresh pork sausages following the addition of linseed oil, fish oil and natural antioxidants. *Meat Sci*. **80**, 1046–54. https://doi.org/10.1016/j.meatsci.2008.04.024

Wood JD, Enser M, Fisher A V., Nute GR, Sheard PR, Richardson RI, Hughes SI, Whittington FM. 2008. Fat deposition, fatty acid composition and meat quality: A review. *Meat Sci*. **78**, 343-358. https://doi.org/10.1016/j.meatsci.2007.07.019